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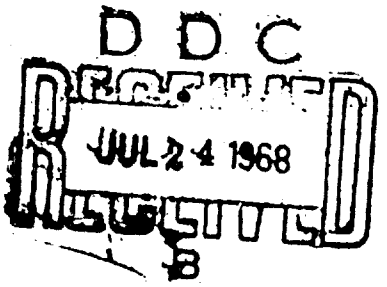

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THE EFFECT OF ETHYLENE ON GROWTH-HORMONES AND GROWTH

(Following is the translation of an article by Hermann Von Guttenberg and Elisabeth Steinmetz, published in the German-language periodical Pharmazie (Pharmacy), Vol. II, No. 17, 1947, pages 17-21. Translation performed by Constance L. Lust.)

Introduction.

Numerous investigations have demonstrated that ethylene has extensive and manifold effects on the growth and development of plants. First, various growth disturbances on germinating plants were observed in "laboratory air" containing ethylene (Wiesner 1878, Neljubow 1901, 1911, Ritcher 1902, 1906, 1912, von Guttenberg 1910). The disturbance expresses itself predominently in a very marked inhibition of linear growth. It is associated with an abnormal thickening of organs and is dependent on a cross-elongation of the parenchymal cells. Simultaneously a loss of geotrophic sensitivity occurs, and this leads to a disorganisation of the organs of the germ. Especially noticeable is the so-called "horizontal-mutation" (Neljubow) of the epicotyls, that is their growth is in almost a horizontal direction. Many of these effects are described in great detail in the book by von Molisch (1937) called "Allelopathie". The pertinant literature of this field is reviewed extensively in this book and we can refer to this book for the present report. For example, we may mention production of epinasty of leaves, forcing of plants from leapshoots, early ripening of fruit, (ethylene produces this itself), inhibition of germination in potatoes, defoliation, proliferation of "lentizellen", and so on. We will discuss several of these phenomena in more detail later in this report.

Since ethylene inhibits linear gro h strongly, it could be postulated that it interferes somehow with the growth hormones of the plant. Knowledge of growth-hormones in general could be expected from this approach. Therefore various researchers have recently tried to answer this question: What effect does ethylene have on the growth hormones of the plant!

Van der Laan (1934) first observed that Avena coleoptile and Vicia faba-epicotyles yield much less growth-hormone when gassed with ethylene in a difussion experiment than do normal plants. He did not observe a disturbance of transport or an elevated consumption of auxin a in the plants which had been treated (gassed) with ethylene. If ethylene gas is conducted by means of an auxin-solution than the growth-material should not lose its effectiveness. Van der Laan concludes from his experiments that the production of growth-hormone is inhibited. Since food materials.

are continuously available and linear growth is stopped by the lack of auxins, thickening results. This problem was later taken up by Michener (1938). He worked with Avena-coleoptile and Pisum vulgare-epicotyles and he obtained rather ambiguous results. In diffusion measurements he found no decrease in auxin production with Avena, however, with Pisum he observed a marked decrease. In transport-experiments with Avena he observed neither a disturbance nor an increased use of auxin. On the other hand he did note both a disturbance of transport phenomena and increased use of auxin with Pisum. In the latter case he assumes auxin is being destroyed. He postulates a gas-dependent sensitivity against growth hormone, but cannot prove this. An important observation is that decapped Pisum-germs react only weakly against entylene, but swell-up noticeable when they are treated with growth hormone. Correspondingly Borgstrom (1939) and Borriss (1943) found that the epicotyles of legumes and the hypocotyles of rye-cockle were less influenced by ethylene after removal of cotyledons. From this it follows that the effect of ethylene occurs primarily on growth hormone. Michener left "growth-hormone-agar" in air containing ethylene for a while and found no change in its activity. But, since he was obviously working with heteroauxin this experiment does not prove anything for natural conditions. Borgstrom (1939) views the effect of ethylene as one of his "transverse reaction" that is, he believes that the polar, linear transport of growth hormone is disturbed, and consequently transverse (lateral) diffusion occurs. This in turn results in a thickening. Hohenstatter (1941) observed an ethylene-effected inhibition of respiration. Following this respiration was elevation in pure air. The sugar-content increases considerably in the gas-atmosphere. Earlier authors have already referred to this. Botjes (1942) extracted Avena coleoptiles, grown in both pure air and in ethylene air, and found a 50% decrease of growth hormone in the latter. He attributed this decrease to increased consumption of this material.

As one can see, the question concerning the manner in which ethylene influences the growth hormone-content of plants is answered very differently by the individual authors, To be sure, the problem is still unsolved.

Methods.

We worked with plants of Avena sativa, Phaseolus coccincus and Helianthus annuus. We cultivated some of the plants in normal air in our dark building and the others in a dark-room of the Institute under glass-cylinders containing ripe apples. The apples served as the source of ethylene. We used coleoptiles of about 2 cm in length; the gas-treated epi-and hypocotyles were also about 2 cm long. The non-gas-treated ones were slightly longer. Phaseolus showed the greatest inhibition. With Helianthus and Avena the effect was less noticeable; they did not thicken but their rate of growth was clearly lessened. With Avena-coleoptile we used the method of Went (1928), but we decapped twice in order to obtain greater angles of bend. The coleoptiles 25-27 mm long) were raised, about 8-10 to a row, in flower pots at 21°C in the dark-house and we observed the reactions with inactive ruby (red)- or orange light.

Results

Diffusion Experiments

When using oat-coleoptiles in diffusion trials we found it advantageous to simple attach the sprouts of both gas-treated and normal plants to the sides of the test-plants. In this manner we obtained larger angles-of-bend with a two-hour diffusate of the sprouts than by using a 1.5% agar plate. As can been seen from table 1 the effect of the gassed sprouts was much weaker than in untreated controls. More noticeable was the behavior of Phaseolus epicotyles. In this case the growth-hormone was obtained, so that the epicotyles were cut off 2 cm under the dome and were hung up in rows in a damp room. At the cut-surface an agarplate was attached and left for two hours. The agar plates of the ungassed epicotyles effected a pronounced bending in the test plants, whereas the gas-treated ones showed no effect (table 1). The angles listed (and those presented later) are averages of 8-10 plants with error of the mean. All experiments were repeated several times.

The experiments' results allow for three interpretations. 1.) The gassed coleoptile produces less growth-hormone than the controls, the gassed epicotyl produces no growth-hormone any more. 2.) The linear transport of growth hormone is inhibited or greatly reduced. 3.) The growth hormone produced is somehow inactivated by the gas. To decide this it was necessary to quantitatively determine the hormone content of gassed plants compared to controls.

However, another diffusion experiment was described earlier. According to Borgstrom the growth hormone of gassed plants should diffuse horizontally into tissues. To test this we attempted to collect the hormone that was diffusing cross-wise. The epicotyl-epidermis was removed at several places with a sharp edge, and in its place was attached a strip of agar. We were unable to collect hormone in this way in all cases, even when the agar strip was touched to all cuts one after another. Accordingly, Borgstrom's explanation appears to be highly improbable.

Extraction Experiments

Extraction experiments were performed in order to determine the growth-hormone cortent. Since Botjes had already reported this for Avena (50% less with gassing) we were content to prove this by extracting Phaseolus. The extraction was done in boiling alcohol for two hours, then evaporated and the residue was taken up in distilled H_2O and agar in the usual way. This agar was then tested on coleoptiles and a very clear-cut result was obtained (table 2). Non-gassed plants contained much and gassed plants contained no (testable) growth hormone. Again the effect is stronger on Phaseolus than on Avena. With oats fewer changes appear than with legumes. This result clearly demonstrates that gassing with ethylene causes a very marked or complete disappearance of growth hormone. This could be caused

Table 1

Diffusion Experiments

Angle of Bend of the Test Plants after 2 hours			
a) Coleoptilesprouts from *Avena*			
Date	Non-gassed Plants	Date	Gassed Plants
11/2/44	$-22.5^{\circ} \pm 2.2^{\circ}$	11/2	$-12.0^{\circ} \pm 1.6^{\circ}$
26/3/44	$-22.5^{\circ} \pm 2.6^{\circ}$	25/3	$-12.5^{\circ} \pm 2.2^{\circ}$
6/6/44	$-35.8^{\circ} \pm 1.6^{\circ}$	6/6	$-19.0^{\circ} \pm 1.4^{\circ}$
b) Epicotyles from *Phaseolus*			
31/10/43	$-19.6^{\circ} \pm 2.3^{\circ}$		
21/1/44	$-26.0^{\circ} \pm 1.6^{\circ}$		
12/2/44	$-21.0^{\circ} \pm 2.0^{\circ}$		no bending
14/2/44	$-26.3^{\circ} \pm 4.2^{\circ}$		

Table 2

Extraction Experiments with Epicotyles of *Phaseolus*

(15-20 plants) test angle after 2 hours

Date	Non-gassed Plants	Gassed-plants
25/10/43	$-16.0^{\circ} \pm 1.2^{\circ}$	
30/10/43	$-22.5^{\circ} \pm 1.8^{\circ}$	no bending

either by suppression of production or by destruction of available materials. Before deciding this the results of several transport experiments will be presented.

Transport Experiments

In these experiments we essentially followed the method of van der Weij (1932, 1934). We used Avena sprouts as a source of growth hormone. The sprouts were closely stacked on 1 mm thick agarplates (1.5%) for two hours. They yielded angles of 20.6°-26.8°. Subsequently 2 mm^2 plates were cut out from this hormone-agar. Four 2 mm long coleoptiles cut out as cylinders were also placed on 2 mm^2 agarplates and were covered with the hormone-containing agarplates. The transport was allowed to proceed in "pure" air for two hours. Then the upper and lower plates were tested. The little cylinders themselves proved to be free of growth hormone. They were removed in part from gassed plants and in part from normal plants. In several experiments we also placed sprouts from normal plants on pre-gassed cylinders or also on non-gassed ones. In this way the ethylene was allowed to act during transport.

The results were always the same. In many cases we found consistently that ungassed cylinders even after 2 hours, allowed so much hormone to pass that the lower plates in the test angle were released by about 20-25°. The upper plates on the other hand showed a decrease of 10-15°. Ungassed little cylinders transported much worse; the lower plates showed 8-14°., and several test plants remained erect. In these experiments a disappearance of hormone occurred even in the upper agar plates; at the end of the experiment an angle of 10-14° was observed. Similar experiments were also carried out with cylinders of hypocotyles of Helianthus Annuus and with epicotyls of Phaseolus. After two hours the upper plate of ungassed plants showed a decrease in the bend of 14-15°, while the lower plates had values of 10-13°. Gas-treated cylinders on the upper plates contained growth hormone corresponding to an angle of bend of 6-10°, even though several test-plants remained erect. The lower plates were effected only weakly in the test, and in many cases not at all. With Phaseolus the result for ungassed cylinders was 11.6° for the upper and 14.4° for the lower. For gassed plants the values were 21.5° for the upper and 0° for the lower.

The result of the experiments is therefore similar; Helianthus took a position between Avena and Phaseolus. The fact that the upper plates of gassed plants lost activity showed that a translocation-inhibition exerted as well as a disappearance of growth hormone. This is in general agreement with Botjes' work who postulated an increased consumption of hormones Another explanation may be that ethylene destroys or inactivates he auxin. It would be possible that the gas causes transformation the plant which make recognition of the auxin impossible under the experimental conditions. Above all it may be postulated that ethylene releases inhibitor which compensate the effect of the growth hormone. In order to elucidate this we did experiments in which the growth hormone itself was gas-treated.

Table 3

Diffusate of Avenasprouts in Agar

Angle of Bend of Test Plants after 2 Hours

Date	Non-gassed Diffusate	Gassed Diffusate	
11/6/44	$-23.5^{\circ} \pm 1.3^{\circ}$	$-8.9^{\circ} \pm 1.3^{\circ}$	
17/6/44	$-25.8^{\circ} \pm 2.3^{\circ}$	$-14.9^{\circ} \pm 2.5^{\circ}$	"Apple-Air"
26/6/44		$-8.1^{\circ} \pm 1.9^{\circ}$	
29/6/44	$-25.5^{\circ} \pm 0.8^{\circ}$	$-9.3^{\circ} \pm 1.5^{\circ}$	"Natural Gas"
1/7/44		$-14.0^{\circ} \pm 2.8^{\circ}$	"Apple-Air"

Table 4

Heteroauxin 1:500,000 in Agar

Angle of Bend of Test Plants after 2 Hours

Date	Non-gassed	Gassed
6/7/44	$-17.0^{\circ} \pm 1.6^{\circ}$	$-18.0^{\circ} \pm 1.9^{\circ}$
7/7/44	$-16.2^{\circ} \pm 1.8^{\circ}$	$-16.6^{\circ} \pm 1.1^{\circ}$

Table 5

Agar with Diffusate of Avenasprouts in a Partial Vacuum

Angle of Bend of Test Plants after 2 Hours

Date	Agar Non-gassed	Agar Gassed	Agar first gassed, then in vacuum
17/11/44	$-20.0^{\circ} \pm 1.7^{\circ}$	$-9.1^{\circ} \pm 1.7^{\circ}$	$-18.6^{\circ} \pm 2.3^{\circ}$
20/11/44		$-8.5^{\circ} \pm 2.6^{\circ}$	$-17.7^{\circ} \pm 2.3^{\circ}$
		3 did not bend	

The Effect of Ethylene on Auxin and Heteroauxin.

Agar-plates containing a diffusate from Avena sprouts were used to study the effect of ethylene on auxin. These were as we have used in earlier experiments. They were placed under glass-cylinders containing "apple-air" (ethylene source), or they were treated with natural gas. The result of these studies was clear-cut as can be seen in table 3. The growth hormone content of the gassed plates was markedly reduced. A few test plants did not react at all anymore. This is not noted in the table. A clear demonstration that auxin was destroyed or inactivated is, nevertheless, still not to be found in the results. It may have been possible that gas could have leaked into the test plants from the agar-blocks and curtailed their reaction and thus curtailed their action. Using gassed agar without hormone would not circumvent this difficulty because then the initiation of bending is removed. Therefore, we used a different approach whereby we made heteroauxin agarplates specifically at a concentration of 1:500,000. The results of the experiment are presented in table 4. It is very clear that the activity of gassed plates was now as great as in ungassed plates. Two facts were unequivocally demonstrated: Ethylene affects auxin either by destroying it or by inactivating it, and in contrast does not affect heteroauxin.

To further confirm this result we repeatedly extracted 200 coleoptiles with boiling distilled water. One-half of the filtered extract was then treated by bubbling natural gas through it for two hours. The other half was concentrated until a residue of 0.3 ml. remained. This was mixed with an equal volume of 3% agar. The gas-treated extract was then also mixed with agar and both specimens were tested. The result at first appeared to contradict the result above, because the gas-treated extract was only a little less active than the ungassed. From this it follows that, that growth-hormone was not destroyed by ethylene, and only the possibility remains that the hormone was somehow reversibly inactivated during the diffusion experiments. Looking at this from a strictly chemical point of view, a destruction of auxin by ethylene is unlikely, since this presupposes a chemical reaction between both compounds. It is more probable that an inactivation occurrs. This blocking of activity could be caused by a loose addition-product of ethylene and auxin. In that case it should be possible to release the auxin again by a gentle treatment as for example by boiling. This assumption proved to be correct. We put growth-hormone-agar plates under a water-jet air pump, where they were kept under partial vacuum for some time. The results (presented in table 5) was unequivocal. Under the partial vacuum the gas evaporated, and the active growth hormone was released. A modification of the earlier-mentioned trial subsequently also gave good results. Since refluxing had destroyed (aufgehoben?=destroyed and/or preserve) the effect of ethylene, we no longer did this. The procedure succeeded when we used alcohol to extract the growth hormone (this time from Coleus internodes). The alcohol was evaporated and the residue was taken up in 0.5 ml. water and only at this time gas-treated. Subsequently the extract was mixed with agar and heated.

gently. Now this material effected either no, or very slight, bending, whereas the non gas-treated extract was highly active. In another experiment we then bubbled natural gas through a 1:500,000 dilution of heteroauxin. A part of this material was then mixed with agar and gave angles of bend of -18.7^{0}-1.4. The ungassed heteroauxin-agar effected values of -18.7^{0}-0.08 (the identical values). In this way it was demonstrated that heteroauxin activity was not blocked by ethylene, in contrast to auxin.

Conclusions.

The main result, ~~or~~ our work points out the fact that auxin is somehow inactivated by ethylene without being destroyed. The experiments to prove this were only carried toward the end of our investigation, since both van der Laan and Michener claimed that ethylene had no influence on growth-hormone in solution or in agar. However, since we reproduced these results in several experiments we feel that our results are valid. Van der Laan worked with a hormone-agar to which he added a gas-treated solution of auxin a. It may be assumed, that he mixed the materials while hot as is usually done. But we know now that by doing this the gas effect was negated. Michener probably used heteroauxin; however, we also found that this is not affected by ethylene. Incidently his angles of bend were so small (about 5 cm.) that differences could hardly be recognized.

We ~~could~~ not find any criteria for the fact that ethylene reduced hormone production. Similarly no alterations appear to be occurring that inhibit the movement of growth hormone. The result of the diffusion-, extraction-, and translocation-experiments is completely understandable if treatment with ethylene inactivates the hormone. ~~It remains~~ to be seen to what extent our findings will help explain the other known effects of ethylene.

Let us first look at linear growth. That this is probably reduced by an inactivation of auxin is obvious. However, one complication already arises with this concept. That is the observation that different kinds of plants behave differently toward ethylene. In the case of Avena only a part of the hormone content disappears; with Helianthus more disappears, and with Phaseolus the total content disappears. Consistent with this is that the changes are more numerous the less the hormone content. Epicotyles of legumes are more deformed than hypocotyles of Helianthus and the coleoptiles are even less altered. Since it is widely believed that growth hormone is the same all over (same chemical formula) it is hard to understand why it is inactivated to different degrees in different plants. It is most probable that other factors also exert an influence on this effect. We may assume this because the removal of auxin alone did not explain observed changes in growth. Merely removing auxin by cutting of the cotyledons, does not

lead to a thickening, only to decreased vertical growth. Also the roots should grow faster before, if auxin is removed. The thickening observed in roots also remains unexplained. It is known that many plants, especially legumes, contain inhibitors, and these may exert an influence in the growth hormone test. Therefore we were not successful in isolating auxins from legumes. In contrast we were able to obtain the material from grasses and other plants. The effect of these inhibitors is overcome by auxin in non-gas treated plants. It is possible that they compensate the remaining auxin activity after ethylene treatment and it is possible that they are somehow responsible for organ thickening. Cotyledons synthesize, or contain, not only the growth hormones, but also the inhibitors. It is thus understandable that germinating plants without cotyledons were inhibited or altered only slightly by ethylene, as was found by Borgstrom (1939) and Borriss (1943). This finding contradicts the assumption that the gas is itself responsible for the thickening, that is to say that the gas is itself an inhibitor.

As already mentioned ethylene effects a complete suppression of geotropism. As a result it comes to an individual motion of the organs corresponding to their structural symmetry. The coleoptile grows as a bilateral symmetrial structure, but often does not grow upright anymore. Dorsal-ventral organs behave differently. They have the inclination to grow on one side. This is clearly seen in leaves which bend epinastically in air containing ethylene. This leave-epinasty was elucidated in our laboratory through several studies. The dorsal side of the leaves contains more auxin than the ventral side, therefore the dorsal side exhibits greater growth. Under the influence of weight the hormone is in part translocated to the lower side. This results in the horizontal position. Dorso-ventral sprouts also react correspondingly. Epicotyle also belong to this group as was clearly shown by Dostal (1941). They orient themselves horizontally when the weight effect is removed because of preferential growth ("horizontal nutation"). When they are under the influence of weight they orient themselves in an upright position, due to auxin translocation. Both van der Laan and Dostal were able to demonstrate more auxin on the convex (upper) side than on the concave during horizontal nutation of coeleoptiles of legumes. If under the influence of ethylene a marked inactivation of auxin occurs (as we have shown), it is possible or even probable, that this is the cause of the horizontal nutation as well as of the leave-epinasty, and all other corresponding movements. In the clinostate these occur because auxin cannot be displaced; in ethylene because of inactivation of auxin. A trial-experiment substantiated this assumption. Coleoptiles which had been decapped and treated with the sprout-diffusate at the cut did not bend when they were exposed to gas in a erect position. However, if the plants were reared in gas-air and then provided with auxin after decapitation the normal bending occurred. From this one may conclude that the absence of bending in ethylene-air may depend on the lack of auxin. It should be noted, however, that leaf-epinasty occurs even in air-containing ether.

Whether ether also inactivates auxin is still to be investigated. It is possible that another factor is involved in both cases of gas treatment. This may be a narcotic effect.

Another effect of ethylene may be explained on the basis of our findings. According to Molisch (1937) ethylene effects defoliation in a very short time. But we know that the stems of leaves also came off if one removes the source of auxin with the cover. If the cover is replaced by an auxin - preparations then they remain intact.

Ethylene has in part a stimulating effect and in part an inhibiting effect in different development processes. It may be used as a forcer for twigs, but on the other hand it inhibits germination of potatoes (Elmer 1932, 1936; Huelin 1932). Furthermore it forces fruits, above all apples into early ripening. In-so far as relations exist between ethylenes and auxin they are unclear, because the function of auxin in the above named processes is unknown. But there can be no doubt that respiration is involved in all these cases. Denny (1924) observed a marked increase in respiration in the ripening of lemons and Elmer (1932, 1936) and Huelin and Barker (1939) found increased respiration in potato tubers. These processes were prevented in the presence of ethylene. Respiration in germs is decreased by ethylene (Hohenstatter, 1923; Harvey, 1913). An increase of sugar is noted during this time. Hohenstatter believes that primarily growth is inhibited and the decrease in respiration is a result of this. We believe that the explanation is forthcoming from another direction. According to Thimann and Commoner (1940) auxin catalyses both growth and the four-carbon acid system of respiration. Therefore, a decrease in auxin should decrease both of these processes. If after removal of ethylene respiration increases sharply this could be explained by the release of auxins together with the accumulation of sugars.

A full treatment of all these questions was not in the protocol of our experiments. We first wanted to explain the relationships between ethylene and auxin. Ethylene is a narcotic and a plasma poison and it undoubtably attacks the protoplasm immediately. Therefore its effect is probably very complex and the solution of the problem will only be forthcoming in a stepwise manner. The effect of ethylene has become very important because we now know that plants themselves can produce the gas. This is also true for indoleacetic acid, the heteroauxins. We found earlier that this material activates auxin and we now found that ethylene inactivates. Maybe these findings will help explain the many puzzling relationships between growth-hormone, growth and development.

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